Aldosterone-Induced Coronary Dysfunction in Transgenic Mice Involves the Calcium-Activated Potassium (BKCa) Channels of Vascular Smooth Muscle Cells

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Background—Cardiomyocyte-specific overexpression of aldosterone synthase in male (MAS) mice induces a nitric oxide–independent coronary dysfunction. Because calcium-activated potassium (BKCa) channels are essential for vascular smooth muscle cell (VSMC) relaxation, we hypothesized that aldosterone alters their expression and/or function in VSMCs.

Methods and Results—Left coronary artery segments were isolated from MAS or male wild-type mice and mounted in a wire myograph. Responses to acetylcholine were assessed (in the presence of a nitric oxide synthase inhibitor) without or with the cyclooxygenase inhibitor diclofenac, the KCa inhibitors charybdotoxin plus apamin, or the BKCa inhibitor iberiotoxin. Expression of BKCa was quantified in hearts by real-time quantitative polymerase chain reaction and Western blot and in isolated coronary arteries by polymerase chain reaction. The effect of aldosterone on BKCa expression also was studied in cultured rat aortic VSMCs. Acetylcholine-mediated coronary relaxation was markedly decreased in MAS mice and was prevented by spironolactone. Diclofenac did not affect the MAS-induced impairment in the responses to acetylcholine, whereas charybdotoxin plus apamin virtually abolished the relaxation in both male wild-type and MAS mice. After iberiotoxin, relaxation to acetylcholine was decreased to a larger extent in male wild-type than in MAS, leading to similar levels of relaxation. BKCa-α and -β1 subunit expressions were significantly decreased in MAS heart and coronary arteries. In cultured VSMCs, aldosterone induced a concentration-dependent decrease in BKCa expression, which was prevented by spironolactone.

Conclusions—Aldosterone overexpression altered VSMC BKCa expression and coronary BKCa-dependent relaxation. The resulting alteration of relaxing responses may contribute to the deleterious effects of aldosterone in cardiovascular diseases. BKCa channels may therefore be useful therapeutic targets in cardiovascular diseases. (Circulation. 2007;116:2435-2443.)

Key Words: aldosterone ■ endothelium-derived factors ■ hormones ■ vasoconstriction ■ vasodilation

Aldosterone antagonists reduce total and cardiac mortality in patients with heart failure.1,2 This benefit may be explained partly by the inhibition of the deleterious effects of aldosterone on cardiovascular system structure and function.3 This is the case for cardiac fibrosis, which is decreased by spironolactone both in experimental models4 and in the treated patients of the Randomized Aldactone Evaluation Study (RALES).5 However, the mechanisms by which aldosterone induces a cardiovascular fibrosis are not fully elucidated. A series of experimental studies indicate that blood vessels are one of the primary targets of aldosterone.6 Indeed, aldosterone induces a vascular oxidative stress that induces a perivascular inflammatory phenotype.5,7,8 These observations are supported by studies in patients with chronic heart failure and standard therapy in whom higher aldosterone levels were associated with systemic evidence of oxidative stress, inflammation, and matrix turnover.9 Similarly, in patients with mild to moderate chronic heart failure treated with an angiotensin-converting enzyme inhibitor, aldosterone blockade improves endothelial function and hence nitric oxide (NO) availability.10 It has recently been demonstrated that aldosterone impairs vascular reactivity by decreasing G6PDH activity.11 Because G6PDH is the main intracellular source of the reduced form of NADPH that serves as a reducing equivalent to limit reactive oxygen species and to maintain levels of reduced glutathione, this observation further explains the induction of vascular oxidative stress by aldosterone.

Clinical Perspective p 2443
To better define the specific cardiac effects of aldosterone, a mouse model has been created in which the aldosterone...
synthase gene is upregulated in cardiomyocytes under control of the myosin heavy chain-α promoter.12 In these transgenic mice, aldosterone is specifically increased in the heart, without changes in its plasma level. Interestingly, the cardiac-specific overexpression of aldosterone synthase induces an increase in the aldosterone concentration in cardiac tissue of 1.7 times relative to controls and a major coronary vascular dysfunction in male aldosterone synthase (MAS) mice without alteration of cardiac structure and function.12 One of the mechanisms that trigger vascular smooth muscle cell (VSMC) relaxation is the activation of potassium channel outward currents, including the large conductance calcium-activated potassium channels (BKCa).13 These channels contain four α subunits forming the pore and four β regulatory subunits. The β1 isorm is smooth muscle cell specific.14,15 Interestingly, BKCa, which is the main potassium channel in human coronary arteries,16 has been shown to be one of the smooth muscle targets for endothelium-derived hyperpolarizing factors (EDHF).9 Moreover, expression of these channels is reduced in spontaneous hypertensin17 or after angiotensin II.18 Because we found in pilot experiments that coronary arteries isolated from MAS mice displayed a significant alteration in NO-independent (presumably EDHF-mediated) relaxations to acetylcholine, we hypothesized that the coronary vascular alteration in these mice involved an impairment in BKCa expression and/or function.

Thus, to obtain more insight into the mechanisms involved in the aldosterone-induced coronary dysfunction, we studied the functional responses of coronary arteries isolated from MAS mice to acetylcholine in the presence of KCa inhibitors. Then, we investigated the BKCa expression in mice hearts and coronary arteries and in isolated rat VSMCs. We demonstrate here that the aldosterone-induced coronary dysfunction of MAS mice involves an alteration of BKCa channel expression and function in VSMCs.

Methods

Animals and Experimental Protocols

All animal experimentation was conducted in accordance with accepted standards of humane animal care as outlined in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Mice from the 8282M line of transgenic mice overexpressing aldosterone synthase in heart have been previously described.12 All control and transgenic mice used in this study for molecular and coronary reactivity experiments were littermates. MAS and male wild-type (MWT) FVB mice were used at 3 to 4 months of age. A group of MAS and a group of MWT mice also were treated with the mineralocorticoid receptor antagonist spironolactone added to drinking water (20 mg · kg⁻¹ · d⁻¹) for 3 weeks.

In Vitro Vascular Studies

Coronary vascular studies were performed as described elsewhere.19 Mice were anesthetized by intraperitoneal injection of a mixture of ketamine (150 mg/kg) and xylazine (6 mg/kg). The heart was removed and immediately placed in cold, oxygenated Krebs’ buffer. A small segment (<1 mm) of the main left coronary artery (diameter, 190 to 220 μm) was carefully dissected and mounted in a small-vessel myograph for isometric tension recording (JP Trading, Aarhus, Denmark). For this purpose, the artery was threaded onto two 25-μm tungsten wires. Normalization procedure was performed after an equilibration period as previously described.19,20 To assess endothelium-dependent NO-indepdendent relaxations, the vessels were pretreated with the NO synthase (NOS) inhibitor N⁶-nitro-l-arginine (LNNNA) (10⁻⁴ mol/L for 30 minutes) before increasing concentrations of acetylcholine (10⁻⁹ to 3 · 10⁻⁷ mol/L) were applied on arteries precontracted with serotonin (10⁻³ mol/L). LNNNA-resistant, acetylcholine-induced relaxations were assessed in the presence of the cyclooxygenase inhibitor diclofenac (10⁻⁷ mol/L), diclofenac plus inhibitors of nitric oxide intermediate, e.g., amin (10⁻⁷ mol/L) and charybdotoxin (10⁻⁷ mol/L), or the specific blocker of BKCa iberiotoxin (10⁻⁷ mol/L). All KCa inhibitor pretreatments were applied for 30 minutes. Endothelium-independent coronary relaxing responses to the NO donor sodium nitroprusside (SNP; 10⁻⁴ to 10⁻⁵ mol/L) also were assessed.

Primary Aortic Smooth Muscle Cells Culture

VSMCs were isolated from 10- to 12-week-old male Sprague-Dawley rat thoracic aorta by enzymatic digestion as described by Capponi et al.21 VSMCs at passages 1 to 2 were cultured in 10% fetal calf serum–Dulbecco’s modified Eagle’s medium and were growth arrested for 24 hours in serum-free Dulbecco’s modified Eagle’s medium before the experiment. VSMCs were incubated for 24 hours at 37°C in Dulbecco’s modified Eagle’s medium supplemented with aldosterone (10⁻¹⁰ to 10⁻⁷ mol/L). Some VSMC cultures were pretreated for 24 hours with spironolactone (10⁻⁴ mol/L) before aldosterone treatment.

Extraction of RNA and Reverse-Transcription Quantitative Polymerase Chain Reaction

Total RNAs from isolated mouse coronary arteries and ventricles and from rat aortic VSMCs were extracted with the RNeasy mini kit (Qiagen, Valencia, Calif) according to the manufacturer’s protocol with a DNase I treatment. The reverse transcriptions were carried out with the Ready-to-go kit (Amersham, Orsay, France) and oligo-dT primer. Primers from genes coding for BKCa-α1 subunit (GenBank accession number: mouse, NM_010610; rat, NM_018128) and BKCa-β1 subunit (GenBank accession number: mouse, NM_031169; rat, NM_019273) were obtained with Primer Express software (Applied Biosystems, Foster City, Calif). Genes coding for the α-smooth muscle actin (α-SMA) (GenBank accession number: mouse, X13297; rat, NM_031004) and for GAPDH (Taquin GAPDH rodent control reagent, Applied Biosystems) were selected as housekeeping genes. Polymerase chain reaction was carried out with Mastermix SYBr Green I (Applied Biosystems) according to the following protocol: 10 minutes of denaturation at 95°C, 30 seconds at each 93°C, and 1 minute at 60°C for 40 cycles (GeneAmp 5700, Applied Biosystems). Dissociation was carried out starting at 60°C. The 2⁻ΔΔCt method, including the normalization to GAPDH, was applied for the quantification. Values of mRNA expression were expressed relative to a cardiac wild-type male RNA pool.

Western Blot of BKCa

MAS and MWT cardiac tissues were homogenized on ice. Purification of membrane-enriched fraction was performed as previously described.22 Protein extracts from brain and liver membrane fractions were used as positive and negative controls, respectively. Afterward, 40 μg protein was denatured for 30 minutes at 37°C in β-mercaptoethanol, separated on 7.5% (for BKCa-α1) or 12% (for BKCa-β1) SDS–polyacrylamide gel, and transferred onto nitrocellulose membrane during 2 hours at 300 mA. After staining with Pierce SuperSignal West Pico kit (Thermo Scientific, Rockford, Ill), the membranes were incubated with IgG anti-rabbit or anti-mouse–coupled horseradish peroxidase (1:10000; Dako, Glostrup, Denmark) in 5% nonfat dried milk. After washes for 5 minutes with Tris buffered saline–Tween 0.1%, the membranes were incubated with IgG anti-rabbit or anti-mouse–coupled horseradish peroxidase (1:5000) and then washed 3 times with Tris buffered saline–Tween 0.1%. Proteins were detected by chemiluminescence (ECL Plus Reagent kit, Amersham Biosciences, Piscataway, NJ) with an Las 3000 (FujiFilm, St Quentin en Yvelines, France).
Histology and Immunostaining

Cryosections (10 μm) from MAS and MWT mouse ventricles were stained with hemalun-eosin and Sirius Red as published. For immunolabeling, cultured cells and ventricular sections were fixed for 10 minutes in methanol at −20°C. After washing in PBS and incubation in blocking solution (5% BSA in PBS), slides were sequentially incubated for 1 hour with rabbit anti–BKCa-β1 antibody (1:100 in 2% BSA in PBS) with FITC-conjugated anti-rabbit IgG (1:50), mouse anti-SMA antibody (1:50), and Texas Red–conjugated anti-mouse IgG (1:100 in 2% BSA in PBS) with FITC-conjugated anti-rabbit IgG (1:50), and Texas Red–conjugated anti-mouse IgG (1:100 in 2% BSA in PBS).

Statistical Analyses

Results are expressed as mean±SEM. For in vitro experiments of coronary function, n represents the number of animals from which the arteries were taken, and concentration-response curves were compared by ANOVA for repeated measures. After ANOVA analysis, Student t test was performed with Bonferroni correction. Values of P < 0.05 were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
Inset, Graphic representation of the values of A at 100% relaxations to SNP (base, 100%; iberiotoxin, 103 mol/L). Indeed, iberiotoxin did not affect the maximal relaxing coronary response in MAS and MWT and reflects an altered BKCa-dependent relaxation in MAS.

In contrast to the response to acetylcholine, iberiotoxin had no marked or significant effect on the response to the NO donor SNP. Indeed, iberiotoxin did not affect the maximal relaxations to SNP (base, 100±1%; iberiotoxin, 103±2%) and induced a small, nonsignificant shift in the sensitivity to the NO donor (IC50, −log M: base, 7.7±0.1; iberiotoxin, 7.4±0.1; n=6; data not shown).

Cardiac and Coronary Expression of BKCa Channel Subunits

Because BKCa-β1 was specifically expressed in VSMCs, we first verified its cellular distribution in ventricles. Figure 4C shows in a representative coronary artery section that the BKCa-β1 immunolabeling was observed in the media of coronary arteries and that there was no labeling in cardiomyocytes. It is noteworthy that because BKCa-β1 is expressed at a very low level, especially in MAS mice, immunolabeling failed to provide evidence of quantitative changes.

We then quantified BKCa mRNA expression in the ventricle of MWT and MAS mice. Figure 5A shows a dramatic decrease in both BKCa mRNA subunits in MAS ventricles (BKCa-α, −75%, P<0.001; BKCa-β1, −50%, P<0.05) with MWT mice.

To verify that the changes in BKCa observed at the level of whole ventricular tissue also are present in the coronary arteries in which functional changes were observed, expression of BKCa-α and BKCa-β1 was measured by reverse-transcription quantitative polymerase chain reaction in isolated coronary arteries (Figure 5B). Similar to measurements obtained in the whole ventricle, our measurements showed that MAS coronary arteries displayed a decrease inBKCa-α (−80%; P<0.01) and BKCa-β1 (−60%; P<0.05).

Ventricular BKCa channel expression was further analyzed by Western blot using anti–BKCa-α and anti–BKCa-β1 subunit antibodies in both membrane-enriched fractions (Figure 6) and total protein extracts (data not shown). The quantitative analysis of membrane-enriched fractions in the MAS group revealed a dramatic decrease in both BKCa-α

Figure 4. Histological analysis of peri-coronary region of MWT and MAS mouse ventricle. Transverse left ventricular cryosections were obtained from MWT and MAS mice. A, Hemalun-eosin staining; B, Sirius Red staining with phase contrast; C, anti–BKCa-β1 antibody labeling. Note that the specific fluorescence in the smooth muscle cells and structure of coronary vessels are similar in MWT and MAS mice. Bar=100 μm.
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Discussion

The present study shows for the first time that an increase in aldosterone concentration in the heart alters coronary vasodilatation by decreasing BKCa expression in vascular smooth muscle cells, leading to decreased BKCa-mediated, endothelium-dependent responses. This work shows that the isolated coronary arteries of MAS mice had decreased acetylcholine-induced relaxation compared with MWT mice. This finding confirms and extends the previous observations made in isolated perfused heart of this strain of mice. Thus, the use of an epicardial left coronary artery segment recapitulates the alterations observed in the whole heart. This observation is important because it allowed us to use the isolated coronary artery to study more in-depth the pharmacological properties of the coronary vasculature of MAS mice. Another advantage of the study in isolated artery segments is that the analysis of endothelium-dependent or -independent responses is not in-

Effects of Aldosterone on BKCa Subunit Expression in Cultured Smooth Muscle Cells

To determine whether aldosterone per se altered BKCa expression, we analyzed its effect in isolated cultured smooth muscle cells. Rat aortic VSMCs were treated with increasing concentrations of aldosterone (10^{-10} to 10^{-7} mol/L) for 24 hours, whereas some VSMCs were pretreated with 10^{-5} mol/L spironolactone for 24 hours before aldosterone. Figure 7A, which represents the immunofluorescent labeling of BKCa in VSMCs, shows that BKCa was present in the cytoplasm with a more intense perinuclear labeling. There was no variation in the intracellular localization of BKCa-β1 after aldosterone (10^{-7} mol/L) or aldosterone plus spironolactone (10^{-5} mol/L).

Aldosterone (10^{-7} mol/L) induced an important decrease in BKCa-α and BKCa-β1 mRNA levels compared with vehicle-treated cells (levels relative to MWT: BKCa-α, 0.4±0.1,
fluenced by possible confounding factors such as changes in coronary reserve, eg, secondary to changes in vascular density, or changes in contractile function that also may affect coronary vascular tone.

The main objective of this work was to understand the mechanism involved in the coronary dysfunction of MAS mice. VSMC relaxation is a complex phenomenon that is controlled by several endothelium-dependent factors, including NO and prostaglandins, as well as EDHFs acting on potassium channels. MAS-induced coronary dysfunction remained unaffected by cyclooxygenase inhibition with diclofenac. This rules out the hypothesis that changes in prostanoid mediators (especially prostacyclin) are involved in MAS-induced coronary dysfunction. This is at variance with observations made previously in rats treated with aldosterone for 3 weeks. Several factors may explain this difference. The concentration of plasma aldosterone was not measured in the rat study, but it is likely to be markedly increased. In our study, the concentration of aldosterone is increased only in cardiac tissue, not in plasma, making it possible that the targets may be different. Moreover, the observations were made on different arteries (coronary versus aorta) from different species (mice versus rats), and it cannot be excluded that the effects of aldosterone differ according to the type of artery.

We found that LNNA/diclofenac-resistant coronary relaxations were virtually abolished by the combination of charybdotoxin and apamin, which blocks the KCa channels involved in smooth muscle hyperpolarization. To further characterize the channel involved, we focused on the role of BKCa channels, which are some of the major channels involved in VSMC relaxation. Besides their responsiveness to EDHF, BKCa channels are activated by L-type Ca\(^{2+}\) channel opening, and the activation of BKCa channels in turn decreases intracellular Ca\(^{2+}\) concentration by closure of voltage-gated Ca\(^{2+}\) channels. Therefore, a reduction in BKCa activity might contribute to impaired relaxation through changes in Ca\(^{2+}\) homeostasis. Indeed, blockade of BKCa channels by the selective BKCa inhibitor iberiotoxin induces a membrane depolarization, followed by an elevation in Ca\(^{2+}\) and vasoconstriction. Interestingly, we observed that iberiotoxin markedly decreased the acetylcholine-mediated relaxation of the isolated coronary arteries of MWT mice, suggesting a major role of BKCa in endothelium-mediated responses to acetylcholine.

In the present experiments, we cannot completely rule out that part of the opening of KCa channels involved in this
response is mediated by residual NO produced despite the presence of a high concentration of a NOS inhibitor.\textsuperscript{27,28} However, we found that iberiotoxin did not affect the responses to SNP, suggesting that it does not inhibit NO-mediated relaxations in our conditions. Thus, the iberiotoxin-sensitive component of the relaxing response to acetylcholine is probably not due to (residual) NO but is most likely mediated by 1 or several EDHFs, different from NO. Remarkably, the amplitude of the inhibitory effect of iberiotoxin was much smaller in MAS compared with MWT mice, resulting in similar relaxation levels after inhibition of BKCa channels in MWT and MAS mice. This result suggests that aldosterone altered specifically the part of the endothelium-dependent VSMC relaxation that is under the control of BKCa channels.

Mice used in our experiments were anesthetized with ketamine, and ketamine has been shown to inhibit BKCa channels in rabbit brain VSMCs.\textsuperscript{29} However, this effect was obtained at a very high concentration (1 mmol/L) and importantly was abolished after washout of ketamine. Our experiments on coronary relaxations were carried out after several washes in buffer during isolation and mounting of arteries and after a 30-minute stabilization in Krebs’ buffer before the addition of drugs to the bath. Thus, in these conditions, we believe that the possible inhibitory effects of ketamine on vascular BKCa, if they exist in vivo, are lost ex vivo during incubation of the arteries.

Our functional experiments demonstrate that cardiac aldosterone synthase overexpression alters BKCa-dependent, endothelium-mediated responses in isolated coronary arteries. Thus, we tested whether such functional changes may be explained by changes in BKCa expression in the heart. Our study demonstrates that both BKCa-\(\alpha\) and BKCa-\(\beta 1\) isoforms were markedly decreased at the mRNA and protein levels in the heart of MAS mice. Thus, we hypothesize that these changes in BKCa in the heart explain the observed alterations in coronary functional responses. The fact that BKCa expression was decreased at mRNA level rules out the major involvement of posttranscriptional pathways. There is strong evidence that the BKCa-\(\beta 1\) isoform is specifically expressed in VSMCs,\textsuperscript{14,15} which was confirmed by our immunolabeling data. Therefore, it may be assumed that alterations in BKCa channel expression using ventricular RNA or protein extracts reflect those occurring in the coronary smooth muscle cells.

The expression of both \(\alpha\) and \(\beta 1\) subunits of BKCa channels also was reduced in isolated coronary arteries. These experiments, together with the immunohistochemical localization, strongly argue for a vascular localization of the observed changes in BKCa. To assess more precisely whether BKCa constitutes a target of aldosterone in VSMCs, we determined the hormone effect in cultured cells. Because of the technical limitation of purifying VSMCs from mouse coronary arteries, we used the well-described preparation of rat aortic VSMCs. The observed aldosterone-induced decrease in both BKCa-\(\alpha\) and BKCa-\(\beta 1\) mRNA expression in rat VSMCs not only confirms the quantitative data obtained in mouse heart but also is in agreement with the functional data obtained in the MAS isolated coronary arteries. Despite the dramatic downregulation of the BKCa-\(\beta 1\) expression in VSMCs induced by aldosterone, there was no difference in the label intensity of this protein as seen on ventricular sections. This is not surprising because it is well known that immunofluorescent labeling is not quantitative. Thus, it emerges from these results that aldosterone regulates the abundance of one of the major VSMC channels, regardless of the vessel type (coronary artery or aorta) and species (rat or mouse). In addition, the fact that the aldosterone-mediated effects on BKCa in VSMCs were prevented by spironolactone indicates that this effect is dependent on stimulation of mineralocorticoid receptors, although the mechanisms by which mineralocorticoid receptors inhibited BKCa transcription are unidentified. The experimental model of cardiac-specific overexpression of aldosterone synthase is obviously different from the physiological situation. Similarly, in severe pathological states such as myocardial infarction\textsuperscript{23} or human heart failure,\textsuperscript{30–32} the levels of aldosterone, either in plasma or in heart, may be far higher than those found in the heart of MAS mice. Nevertheless, because of the moderate and tissue-specific increase in aldosterone, the MAS mice model provided evidence of an effect of aldosterone that would likely have been difficult to detect in classic experimental models or in patients. With this specificity taken into account, our results lead us to propose that the beneficial outcome of long-term treatments with aldosterone antagonists may be mediated, at least in part, through the regulation of the expression of BKCa channels in VSMCs.

Our results support the hypothesis that in pathological states with increased aldosterone concentration, defects in arterial relaxation might involve a BKCa channel downregulation in artery media. Interestingly, downregulation of the BKCa-\(\beta 1\) subunit has been reported in spontaneously hypertensive rats.\textsuperscript{17} Moreover, recent experiments showed that BKCa-\(\beta 1\) also was reduced in mice with angiotensin II–induced hypertension and after in vitro incubation with angiotensin II by a mechanism involving calcineurin/NFATc3 signaling.\textsuperscript{18} Although to the best of our knowledge a similar effect of aldosterone has not been reported, it is possible that aldosterone may contribute to the observed effects of angiotensin on BKCa, at least when administered in vivo. The relationship between aldosterone and vascular BKCa channels is further supported by the observation that BKCa channel–deficient mice display an increased blood pressure linked to impaired vasodilatation, together with primary hyperaldosteronism.\textsuperscript{33} Despite the fact that the mechanism of induction of the hyperaldosteronism in these mice is unknown, all these observations converge toward a major role of BKCa channels in the control of blood vessel properties.

One of the limitations of the present study is that, although we have evaluated the functional consequences (impaired relaxation) and the changes in expression of the BKCa channel, we have not directly assessed whether these changes in expression translate into changes in the activity of BKCa in the coronary arteries, although previous experiments established a link between the alterations of BKCa channel expression and that of their activity.\textsuperscript{34} In any case, further studies using, for example, the microelectrode technique adapted to mice coronary arteries\textsuperscript{35} are required to precisely assess the exact electrophysiological changes associated with
aldosterone overexpression. In addition, our study concentrated on the mechanisms of the impaired smooth muscle relaxation and was not designed to determine the exact nature of the endothelial mediators (presumably EDHF) involved, for which further studies also are required.

Conclusions
We have documented a specific pattern of changes in BKCa subunits in the VSMCs of animals submitted to increased endothelium-dependent, BKCa-mediated coronary relaxations. BKCa channels may thus represent a therapeutic target of interest in diseases such as hypertension or heart failure. The concept of aldosterone-mediated alterations of (coronary) vascular tone mediated by changes in BKCa channel expression brings new insights into the mechanisms leading to vascular dysfunction in cardiovascular diseases and may help to identify new therapeutic targets in this setting.

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Aldosterone antagonists reduce total and cardiac mortality in patients with heart failure. This benefit may be explained partly by the inhibition of deleterious effects of aldosterone such as fibrosis on the cardiovascular system. However, the mechanisms of aldosterone action, particularly in the heart, remain partly unclear. Experimental and clinical observations point to the vessels as primary targets of aldosterone, where it induces oxidative stress, leading to altered vascular reactivity and a perivascular inflammatory phenotype. These alterations occur when aldosterone is strongly increased in plasma or in tissue such as in heart failure or after myocardial infarction. To explore the effects that aldosterone may have in the heart at concentrations slightly above normal, we used transgenic mice that have a cardiomyocyte-specific upregulation of aldosterone synthase. We have previously shown that these mice have a moderately increased cardiac concentration of aldosterone (1.7 times that of controls), which results in an abolished coronary reserve in males with unaltered cardiac structure and function. The present study demonstrates that aldosterone altered the coronary vasodilatation by decreasing the expression of the main repolarizing potassium channel (BKCa) of coronary vascular smooth muscle cells. This led to decreased BKCa-mediated, endothelium-dependent responses. Thus, this study suggests that in preclinical states with slightly increased aldosterone, hitherto undetected alterations of the vascular BKCa potassium channels leading to altered coronary response to physiological stimuli may occur. This possibility reinforces the interest in aldosterone antagonists and suggests that BKCa channels may represent a therapeutic target in these situations.
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